REMARKS

The Applicants greatly appreciate the Examiner's careful attention to this application. Claims 1-10, 12, 14-23, 24 (as directed to a virus), 25, 27-35 and 37 were pending at the time the Office Action was issued. All other claims were deemed withdrawn as directed to non-elected species.

Objections to the Specification

Title

The Examiner objected to the title of the invention. Applicant has requested the title be amended as suggested by the Examiner.

Table 8

The Examiner objected to the Table contained on pages 42 and 43 of the present application (with reference to the PCT Publication) as "illegible" and being identified as both Table 4 and Table 8. Applicants have requested that the table on pages 42-43 of the present application be replaced with revised Table 8 which addresses the Examiner's concerns.

Trademark Usage

The Applicant has amended the specification to correct use of the certain trademarks as noted by the Examiner.

Sequence Rules Compliance

Tables 2 and 3 on pages 35-39 of the present application (with reference to the PCT Publication) contained nucleotide sequences without corresponding SEQ ID numbers. The nucleotide sequences in Tables 2 and 3 were however included in the sequence listing provided upon filing. Those tables have been revised to include the appropriate SEQ ID numbers and replacement sheets are submitted herewith. Specifically, an extra column has been added to each Table reflecting the SEQ ID of each nucleotide sequence. Applicant has requested revised Table 2 and 3 be incorproated into the specification and Tables 2 and 3 as filed be removed.

Claim Rejections/Objections

Claim objections

Claim 1 was objected to for the inclusion of a "stray underline mark." That mark has been deleted.

35 U.S.C. § 112, 2nd rejections

Claim 22 was rejected for including the limitation "enhancement primers." Claim 22 has been amended and the term "enhancement" has been replaced with "enrichment." Accordingly, claim 22 now contains a limitation of having three or more pairs of target enrichment primers. As amended, claim 22 is fully supported by the current specification. *See* pg. 10, ll. 23-28.

Claim 25 was rejected for including the term "including." That term has been deleted from claim 25. Further, a new claim, claim 89, has been added that properly depends from claim 25. Support for new claim 89 is found at pg. 26, ll. 29-35 and pg. 27, ll. 1-5 of the current specification.

35 U.S.C. § 102 rejections

Claims 1-10, 12, 14-22, 27 and 29-34

Claims 1-10, 12, 14-22, 27 and 29-34 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 7,262,030 issued to Chen (Chen '970). The Applicant respectfully traverses the Examiner's rejection.

Chen '970 must teach each and every limitation of those claims to be anticipatory. *See* MPEP 706.02(V). As discussed in detail below, Chen '970 does not teach the limitations of independent claim 1 and is therefore not anticipatory. Further, since Chen '970 does not teach each and every limitation of claim 1, it by definition, cannot teach all of the limitations of claims 2-9, 12, 14-22 and 29-34 which properly depend from claim 1.

Chen '970 teaches a high throughput method of single nucleotide polymorphism (SNP) sequencing and genotyping. Through a series of polymerase chain reaction (PCR) procedures, various sequencible and ligatible structures are produced. Chen '970 does not teach a "second pair of target enrichment primers" that "bracket" a target sequence as required by the limitation of claim 1(a)(iii). As shown in Figures 1 and 2A of Chen '970, only one pair of primers are taught therein bracket the target sequence "X." Further, as explained at col. 7, ll. 11 and 19-28

> [a]s can be seen, in FIG. 1, the inner primer set is composed of primers P1 and P2, and the outer primer set is composed of primers P1 and P3. (In this example, Primer P1 is utilized in both primer sets.) The P1-P3 outer primer set flanks the indicated target site whereas the P1-P2 inner primer pair does not. PCR amplification of the DNA with these primers will produce two amplicons. The larger of the two is the outer amplicon formed by amplification with the P1-P3 pair; this amplicon includes the target site. The smaller of the two amplicons, the inner amplicon, contains base pairs which lie within the bounds of the larger outer amplicon (i.e. is "nested" within the larger amplicon), but does not contain the target site. The ultimate product of the PCR reaction is double strand DNA of two lengths, only one of which (the longer) contains the target site, and the shorter of which contains a subset of the bases contained in the longer strand.

The lack of the second pair of target amplification primers that "bracket" a target sequence as taught in Chen '970 is by design. The goal of Chen '970 is use the 3 primers illustrated to produce an outer amplicon containing the target sequence and an inner amplicon which excludes the target sequence, the two amplicons forming at least one of (i) a ligatable structure which includes a 3'-5' sequence which excludes the target sequence hybridized to a 5'-3' sequence which includes the target sequence and (ii) a sequencible structure which includes a 5'-3' sequence which excludes the target sequence hybridized to a 3'-5' sequence which includes the target sequence (see column 3 lines 30-53 for example and the figures). The use of a second pair of target enrichment primers that bracket the target site would destroy the utility of the methods disclosed in Chen '970. As such Chen '970 teaches away from the use of the primer configurations taught and claimed in the current application.

Additionally since Chen '970 does not teach the second pair of target enrichment primers contemplated by claim 1, it cannot teach one of the second pair of target enrichment primers that bracket a target sequence and having a 5' end binding tag which corresponds to the sequence of one of a pair of target amplification primers and the other of the second pair of target enrichment primers comprising at its 5' end a binding tag corresponding to the sequence of the other of said pair of target amplification primers as required by claim 1(a)(iii).

As discussed in detail above, Chen '970 fails to anticipate each and every limitation of claim 1; therefore it cannot anticipate claims 2-10, 12, 14-22, 27, 29-34 which properly depend from claim 1. *See* MPEP 608.01(n) (explaining that a proper dependent claim contains all of the limitations of the claim from which it depends).

Applicant respectfully requests the Examiner withdraw the present rejection.

Claim 37

Independent claim 37 was rejected under 35 U.S.C. § 102(e) as anticipated by U.S. Patent Application No. 10/231,381 by Chen (Chen '381). The Applicant respectfully traverses the Examiner's rejection.

Applicant respectfully suggests Chen '381 does not anticipate claim 37. As an initial matter, Applicant would like to point out the difference between the primer configurations of Chen '381 and the instant application. In Chen '381, the primary primers designated 10 in FIGS. 1 and 2 are equivalent to the second set of target enrichment primers (also designated F_{in} and R_{in}) of claim 1 (and the claims dependent therefrom). The primary primers of the Chen '381 comprise a specificity domain (designated 11) and an artificial domain (designated 12). The specificity domain contains an allele element (designated 13) on the 3' end and a target element (designated 14) on the 5' end. The artificial element contains a coupling element (designated 15)

and a connecting element (designated 16); it should be noted that the coupling element 15 is distinct for each given allele (see paragraphs 0032, 0041 and 0042). Therefore, the artificial element 12 is distinct for each allele target. This distinctness leads to problems in designing multiplex PCR reactions (such as differences in T_m). Removing this variability in the artificial element would destroy the utility of the approach described in Chen '381. In this regard, Chen '381 teaches away from the methods described and claimed by the Applicant.

In contrast, the second set of target enrichment primers in the current specification contain first and second binding tags that do not contain an equivalent "variable sequence" corresponding to the coupling element 15 in Chen '381. The first and second binding tags are distinct from one another; however, the first binding tag and the second binding tag are identical for each and every target sequence amplified. This feature provides an advantage in the amplification reactions described and is not taught or suggested by Chen '381.

Likewise, the portion of the first set of target enrichment primers that bind the nucleic acid containing the target sequence do not contain a 3' mismatch as do the primers of Chen '381.

The Chen '381 simply states that multiple primers can be used to detect and differentiate between different allelic variants of a nucleic acid sequence; however, each of the primer sets binds to the same location on the nucleic acid and binds to the same target sequence (excepting the site of the allelic variation) (see FIGS 1 and 2 and paragraphs 0032-0052).

Applicant respectfully requests the Examiner withdraw the present rejection.

35 U.S.C. § 103

Claims 1-10, 12, 14-23 and 27-34

Claims 1-10, 12, 14-23 and 27-34 were rejected under 35 U.S.C. § 103(a) as obvious over the Chen '381, U.S. Patent No. 5,811,235 to Jeffreys (Jeffreys) in view of U.S. Patent No. 5,314,809 to Erlich (Erlich) and a Qiagen publication (the Qiagen Reference). Applicant respectfully traverses the Examiner's rejection.

As discussed in great detail above, there are many differences in Chen '381 and the methods defined by the claims of the current application; those arguments are incorproated herein by reference. One of ordinary skill in the art would not have been motivated to alter the primers disclosed in Chen '381 to arrive at the Applicant's claims since such alternation would destroy the utility of the methods described in Chen '381 and result in an inoperable invention.

In addition, Chen '381 does not teach or suggest then beneficial properties of the first set of target enrichment primers in combination with the second set of target enrichment primers as disclosed by Applicant. As stated in the specification, the first set of target enrichment primers provide for surprisingly superior amplification of the target sequence. Such an effect was not

previously noted in the prior art. The applicants submit the data in attached Tables 1A and 1B and 2 indicating the increased amplification efficiency of the disclosed methods when the first set of target amplification primers are added.

Table 1 shows the results from a respiratory panel using the methods of the present disclosure. The reaction was performed essentially as described in Example 5 of the current specification with the exception that in Table 1B the first set of amplification primers were omitted. The rows indicate the identity of the nucleic acid used in the amplification step and the columns indicate the identity of the detection oligonucleotide used (the reaction mixture contained primers for all target sequences to be detected). As can be seen in a comparison of Table 1A and Table 1B, the omission of the first set of target enrichment primers significantly diminished the ability of the second set of amplification primers and the target enrichment primers to amplify the desired target sequence for detection. For example, in row 2 (RSVA), the inclusion of the first set of target amplification primers resulted in a detection value of 1837; this value was reduced to 836 when the first set of target enrichment primers were omitted.

The unexpected results are even more striking when the concentration of nucleic acid used as the template is decreased as shown in Table 2. The reaction was performed essentially as described in Example 5, with the exception that in one set of reactions (indicated by dark gray shading) the first set of target enrichment primers were omitted. As above, the rows indicate the identity of the nucleic acid used in the amplification step and the columns indicate the identity of the detection oligonucleotide used (the reaction mixture contained primers for all target sequences to be detected). In Table 2, the B-samples indicate unknown clinical isolates obtained from a major southeastern research university. The legend below Table 2 indicates the occurrence of the genes to be detected in each sample as indicated by + or -. As can be seen in Table 2, the omission of the first set of target enrichment primers significantly diminished the ability of the second set of amplification primers and the target enrichment primers to amplify the desired target sequence for detection. The effect was greater when the concentration of nucleic acid template was reduced from 1.0 ng to 0.1 ng.

Applicant submits that Chen '381 does not teach or suggest the configuration of the second set of target enrichment primers or the placement and use of the first and second set of target enrichment primers. Therefore, the applicant respectfully suggests that Chen '381 does not render claim 1 obvious. Further, claims 2-9, 12, 14-23 and 27-34, all of which depend from claim 1, are not rendered obvious by the Chen '381.

Regarding claims 1-3 and 28, the Examiner cited Jeffreys as teaching a method for multiplex primer-based amplification of a target sequence from a plurality of agents, said target

sequence being different for each agent. The Examiner specifically pointed to Figures 15 and 27 of the Jeffreys and Example 15 for providing the limitations of claim 1. Applicants respectfully suggest that the Jeffreys does not teach the limitations of claim 1 and that, as discussed above, the novel primer configuration of the current specification offers many advantages over Jeffreys (and Chen).

Jeffreys teaches a method of characterizing genomic DNA using primers that "selectively prime" internal repeats of a tandemly repeated region. See col. 1, ll. 5-10. Representative methods of Jeffreys teach the use of one or more type specific primers (each of the type specific primers hybridizing to the same repeated sequence) to initiate an amplification reaction to produce a first template. The type specific primers comprise a tail sequence that does not hybridize to the repeated sequence. One or more common primers bind the first template and initiate amplification back towards the type specific primers, resulting in amplification of the tail sequence as well. In certain embodiment, the tail sequence primers then initiate amplification of the sequence incorporating the type specific primers and the tail sequence. The tail specific primers and the common primers are preferentially used at higher concentrations that the type specific primers.

Jeffreys does not teach a method that uses primer combinations to bracket a target sequence as described in claim 1. Claim 1 requires a second set of target enrichment primers, each having binding tag that provides a binding site for the target amplification primers. The method of Jeffreys provides a method in which only 1 primer (the type specific primer) is used to flank one side of the target sequence and multiple primers (the common primers) can be used to flank the other side of the target sequence.

Example 15 (represented in Figs. 15A-15C) of the Jeffreys does not teach the use of a second pair of target enrichment primers as required by the limitations of claim 1. In Example 15, there are four (4) primers present in the first PCR reaction step; however, only two of those primers, the ARMS primers, are specific to the target, in this case a mutation in the APC gene. *See* col. 35, ll. 46-67. More specifically, there is no express or suggested teaching that the control primers bracket the target; as discussed above, the specification and Fig. 27 suggest that this is not the case. Further, the control primers would not cause any amplification of the target sequence. *See* col. 36, ll. 1-14 of the Jeffreys (discussing the concept of a positive control in that in the absence of the target mutation, only the extension product of the control primers is present therefore indicating that the control primers do not amplify the target). In the current specification, the first and second pairs of the target enrichment primers used at low concentrations provide amplification of the target sequence (albeit limited). *See* pg. 10, ll. 6-9.

The Jeffreys does not teach the novel primer configuration of the current specification. Further, as discussed in great detail above, as regards to attached Tables 1A and 1B and 2 indicating the increased amplification efficiency, the Jeffreys does not teach or suggest the advantages of the novel primer configuration.

Example 18 (shown in FIG. 27) provides a similar approach. As described in the methods section, primers 31A (common) and Tag (tail specific primer) are added at 1uM and either of primers 31-Tag-A or 31-Tag-G (common primer) are added at a higher concentration. *See* col. 52 ll 10-43). As such, there is not a second pair of target enrichment primers flanking the target sequence. Fig. 27 shows a number of alternative common primer binding sites, but only one is used in order to carry out the methods described. The use of multiple common primers for a single target sequence would complicate the resolution of the amplification product sizes, which is used to determine the coding of the results, thereby defeating the purpose of the methods described by Jeffreys.

In addition, FIG. 27 shows that the Tag primer described is not a universal primer as described in the Applicant's claims. Fig. 27 clearly shows that the 5' base of the Tag primer corresponds to the 3' base of the 31-Tag-A/G primer. As the type specific primer will be different based on target sequence, the Tag primer will also be required to be different.

The Examiner cites Erlich for the detection of the AIDS virus by certain methods. The Examiner cites the Qiagen Reference as disclosing certain lengths for standard primers. Therefore, Erlich and the Qiagen Reference do not, individually or collectively, cure the deficiencies noted above in Chen '381 or Jeffrey.

Applicant respectfully requests the Examiner withdraw the present rejection.

Claims 24 and 25

Claims 24 and 25 were rejected over the (1) Chen '381 or (2) the Chen '970, Jeffreys, the Qiagen Reference and Elrich in view of Elnifro. As discussed above, neither Chen'970 or Chen '381 teach or suggest the subject matter disclosed by Applicant in claim 1, from which claims 24 and 25 depend. Furthermore, Chen'381, Chen '970, Jeffreys, Erlich and the Qiagen Reference have been discussed above with regard to claim 1 and claims dependent thereon.

The Examiner cites Elnifro for the teaching of detecting influenza A and B virus by multiplex amplification. Therefore, Elnifro does notcure the deficiencies noted above in the cited references.

Applicant respectfully requests the Examiner withdraw the present rejection.

Claim 35

Claim 35 was rejected as obvious over the Chen '970 as applied to claims 1, 30 and 32 in view of US Patent 5,194,300 (Cheung). As discussed, the Chen '970 does not teach or suggest the subject matter disclosed by Applicant in claim 1, from which claim 35 depends.

The Examiner cites Cheung for teaching the use of fluorescent microspheres for detection of DNA. Therefore, Cheung does not cure the deficiencies noted above in Chen '970.

Applicant respectfully requests the Examiner withdraw the present rejection.

Claim 35

Claim 35 was rejected as obvious over the Chen '381, Jeffrey, Erlich and the Qiagen Reference as applied to claims 1, 30 and 32 above, in further view of Cheung. As discussed, the Chen '381, Jeffrey, Erlich and the Qiagen Reference do not, individually or collectively, teach or suggest the subject matter disclosed by Applicant in claim 1, from which claim 35 depends.

The Examiner cites Cheung for teaching the use of fluorescent microspheres for detection of DNA. Therefore, Cheung does not cure the deficiencies noted above regarding Chen '381, Jeffrey, Erlich and the Qiagen Reference.

Applicant respectfully requests the Examiner withdraw the present rejection.

CONCLUSION

Applicants respectfully request the Commissioner of Patents consider the enclosed remarks and enter the following submission into the record, in response to the Examiner's Office Action dated 08/05/2009. If the Examiner requires additional action that may benefit from a telephone call, Applicants invite a call to their attorney of record, T. Gregory Peterson (Reg. No. 45,587). E-mail correspondence and transactions to gpeterson@babc.com are authorized and encouraged.

Applicant has diligently sought to comply with all requirements. The Application is believed to be in condition for allowance, and a timely Notice of Allowance is respectfully requested.

Respectfully submitted,
BRADLEY ARANT BOULT CUMMINGS LLP

11-4-09
Date

T. Gregory Peterson
Reg. No. 45,587

Appendix A
Replacement Sheets 42, 43 and 43a

Table 8- The sensitivity of the assay system

Sample/Target		ADV	CPN	MPN	INFA	INFB	P1V1	P1V3	RSV	SARS1	SARS2	SARS3	ENT
1	RT-PCR Blank	8	35	7	21	37	16	85	16	11	5	14	9
2	Serum only	12	40	11	29	37	10	71	9	8	6	9	9
3	Serum only	15	32	7	24	34	13	76	9	6	4	11	7
4	Serum only	11	31	6	27	28	19	85	8	10	5	11	10
5	ADV4 10e3 ctrl	976	38	15	32	22	20	76	6	7	9	19	18
6	ADV4 10e3 #1	486	45	12	36	20	18	82	10	9	14	185	24
7	ADV4 10e3 #2	112	42	6	31	24	25	93	13	23	20	16	19
8	ADV4 10e3 #3	105	40	20	27	20	26	81	16	9	10	6	17
9	ADV4 10e2 #1	122	41	20	35	38	27	91	18	14	12	16	23
10	ADV4 10e2 #2	162	42	16	28	23	27	54	23	10	15	16	18
11	ADV4 10e2 #3	35	42	12	38	23	22	67	8	15	10	7	2
12	ADV4 10e1 #1	31	39	15	32	29	26	82	8	15	16	13	22
13	ADV4 10e1 #2	25	33	23	38	16	26	63	20	18	13	24	14
14	ADV4 10e1 #3	25	28	22	25	10	28	59	21	21	18	10	11
28	CPN 10e4 ctrl	23	362	17	34	24	30	66	18	11	14	16	45
29	CPN 10e4 #1	10	256	12	33	24	26	73	18	14	15	13	9
30	CPN 10e4 #2	18	111	9	34	27	24	78	7	13	13	22	13
31	CPN 10e4 #3	16	239	8	24	19	21	75	17	19	9	17	32
32	CPN 10e3 #1	16	103	13	31	26	26	88	11	17	8	9	18
33	CPN 10e3 #2	14	43	12	41	23	25	91	14	10	12	13	9
34	CPN 10e3 #3	15	85	12	35	15	27	79	14	10	17	17	7
35	CPN 10e2 #1	11	33	9	28	19	18	56	15	10	6	7	19
36	CPN 10e2 #2	6	35	8	34	23	26	53	13	11	10	6	12
37	CPN 10e2 #3	19	42	11	24	22	20	63	9	12	10	11	19
38	CPN 10e1 #1	11	42	10	31	13	17	65	4	10	7	13	17
39	CPN 10e1 #2	22	32	17	25	20	24	47	14	15	13	13	4
40	CPN 10e1 #3	13	32	13	29	17	25	41	12	8	8	13	17
41	MPN 10e4 ctrl	12	34	578	29	32	19	75	11	8	12	6	11
42	MPN 104 #1	13	33	262	24	39	12	76	12	6	10	9	6
43	MPN 104 #2	9	28	354	21	17	15	57	12	8/	4	12	9
44	MPN 104 #3	12	30	428	23	18	15	55	8	4	4	15	12
45	MPN 103 #1	14	34	109	22	21	12	88	11	13	12	7	7
46	MPN 103 #2	11	37	23	28	19	17	95	9	13	10	14	6
47	MPN 103 #3	8	37	82	30	22	12	72	7	7	7	8	9
48	MPN 102 #1	6	54	9	36	21	21	124	12	10	11	7	17
49	MPN 102 #2	12	42	18	28	22	7	96	9	1	13	13	10
50	MPN 102 #3	13	39	19	29	18	10	106	17	7	4	9	11
51	MPN 101 #1	8	38	11	26	21	16	101	16	4	3	14	0
52	MPN 101 #2	11	38	9	32	26	17	102	10	5	12	11	10
53	MPN 101 #3	13	34	15	31	19	11	91	14	12	6	11	10
54	INFA 10e4 ctrl	16	35	16	840	56	14	92	17	16	12	13	17
55	INFA 10e4 #1	20	38	18	322	38	21	83	17	9	7	9	13
56	INFA 10e4 #2	17	33	14	339	43	15	92	14	13	11	10	17
57	INFA 10e4 #3	11	45	19	478	42	23	101	48	11	14	17	21
58	INFA 10e3 #1	14	46	10	103	20	15	67	16	5	8	15	8
59	INFA 10e3 #2	20	41	11	113	26	11	83	18	9	15	6	13
60	INFA 10e3 #3	11	45	11	206	25	14	76	17	7	13	11	17
61	INFA 10e2 #1	12	26	7	49	15	17	47	12	5	8	11	21
62	INFA 10e2 #2	14	21	6	97	24	22	47	18	13	5	18	16

63	INFA 10e2 #3	15	32	7	90	18	18	38	20	4	6	15	12
64	INFA 10e1 #1	12	27	14	36	3	19	48	14	12	16	6	13
65	INFA 10e1 #2	16	26	12	31	14	20	41	14	12	7	9	9
66	INFA 10e1 #3	23	37	12	28	23	13	40	12	16	11	17	19
67	INFB 10e4 ctrl	17	24	11	35	349	14	47	10	10	13	13	7
68	INFB 10e4 #1	11	32	10	36	174	21	48	15	8	12	14	19
69	INFB 10e4 #2	12	30	5	32	266	19	56	10	11	9	8	4
70	INFB 10e4 #3	13	28	13	26	277	20	53	12	15	15	13	12
71	INFB 10e3 #1	13	31	15	20	20	16	61	14	12	15	10	10
72	INFB 10e3 #2	10	30	11	22	20	19	43	13	12	6	12	6
73	INFB 10e3 #3	16	35	14	29	21	22	53	9	2	15	16	13
74	INFB 10e2 #1	12	29	11	24	22	17	50	16	12	6	13	18
75	INFB 10e2 #2	15	28	8	19	22	19	65	15	11	16	17	13
76	INFB 10e2 #3	20	38	18	31	23	17	67	8	12	5	11	16
77	INFB 10e1 #1	11	35	7	30	44	12	76	8	18	5	9	14
78	INFB 10e1 #2	19	44	13	28	42	14	96	13	2	8	12	7
79	INFB 10e1 #3	18	30	4	28	25	17	69	12	6	3	16	14
80	PIV-1 10e4 ctrl	12	69	16	23	33	980	94	15	7	12	20	13
81	PIV-1 10e4 #1	16	54	8	20	18	461	47	8	17	5	18	9
82	PIV-1 10e4 #2	17	42	11	21	22	555	40	8	12	9	15	6
83	PIV-1 10e4 #3	14	32	10	14	16	630	38	10	9	17	12	6
84	PIV-1 10e3 #1	10	31	8	13	13	145	39	9	10	10	13	4
85	PIV-1 10e3 #2	22	46	6	13	13	149	54	13	19	11	15	16
86	PIV-1 10e3 #3	12	35	12	20	10	98	52	12	15	10	15	12
87	PIV-1 10e2 #1	15	40	6	16	4	26	37	6	7	12	13	9
88	PIV-1 10e2 #2	17	44	11	20	11	27	57	9	13	9	9	10
89	PIV-1 10e2 #3	22	38	13	18	10	35	57	12	9	9	18	9
90	PIV-1 10e1 #1	14	41	16	22	15	19	56	16	15	3	10	9
91	PIV-1 10e1 #2	9	46	14	24	14	20	45	10	11	13	13	13
92	PIV-1 10e1 #3	9	41	5	11	18	22	53	15	10	15	12	11
93	PIV-3 10e4 ctrl	12	54	16	30	33	25	1241	16	11	15	13	15
94	PIV-3 10e4 #1	12	58	5	29	18	31	290	21	8	7	11	5
95	PIV-3 10e4 #2	22	62	20	42	22	33	272	14	20	15	20	25
96	PIV-3 10e4 #3	13	49	8	30	16	21	229	13	7	0	10	21
97	PIV-3 10e3 #1	17	67	9	34	13	14	214	15	8	18	12	17
98	PIV-3 10e3 #2	18	70	15	39	13	21	301	11	9	7	11	11
99	PIV-3 10e3 #3	12	64	21	40	10	32	265	15	11	18	15	23
100	PIV-3 10e2 #1	17	67	16	23	4	29	171	15	20	10	17	7
101	PIV-3 10e2 #2	21	67	8	31	11	21	189	13	21	6	17	6
102	PIV-3 10e2 #3	16	67	9	36	10	36	181	19	10	13	9	15
103	PIV-3 10e1 #1	12	76	14	34	15	30	190	8	12	10	9	11
104	PIV-3 10e1 #2	13	70	14	32	14	35	215	21	4	8	11	15
105	PIV-3 10e1 #3	19	26	13	32	18	33	36	13	11	10	19	12
106	RSVB 10e4 ctrl	19	47	7	16	25	16	82	1232	11	9	15	18
107	RSVB 10e4 #1	11	47	16	32	31	18	93	1154	7	9	11	24
108	RSVB 10e4 #2	13	48	14	25	33	22	91	905	10	9	12	16
109	RSVB 10e4 #3	13	39	14	25	21	21	76	948	5	8	9	15
110	RSVB 10e3 #1	6	38	17	28	14	26	73	149	19	17	15	20
111	RSVB 10e3 #2	16	42	6	21	21	20	76	217	11	15	19	16
112	RSVB 10e3 #3	14	42	8	29	32	28	84	198	6	9	12	24
113	RSVB 10e2 #1	11	44	16	33	29	17	100	40	14	16	7	21
114	RSVB 10e2 #2	6	39	16	23	21	21	88	23	15	9	11	14
115	RSVB 10e2 #3	20	41	12	20	36	22	90	29	9	17	13	25

116	RSVB 10e1 #1	23	49	10	29	30	18	95	12	16	11	7	17
117	RSVB 10e1 #2	15	44	15	27	35	17	94	11	11	7	5	18
118	RSVB 10e1 #3	13	54	20	35	33	24	91	23	10	16	9	16
119	SARS 10e4 ctrl	34	34	13	14	33	27	34	15	2121	1686	344	19
120	SARS 10e4 #1	33	29	12	18	36	19	30	17	2066	1742	337	14
121	SARS 10e4 #2	36	27	15	21	32	29	31	18	1514	1438	274	18
122	SARS 10e2 #1	44	37	16	17	31	33	31	20	1245	1316	252	13
123	SARS 10e3 #2	27	28	14	14	24	25	33	17	930	1069	180	11
124	SARS 10e2 #1	53	27	17	13	20	28	30	17	479	664	119	12
125	SARS 10e2 #2	42	24	13	21	17	27	29	15	196	381	53	9
126	SARS 10e1 #1	46	22	8	17	17	24	36	15	88	168	37	15
127	SARS 10e1 #2	10	16	14	23	10	17	28	10	6	4	5	18
128	RhV 10e4 ctrl	7	31	10	22	25	19	76	10	10	9	9	838
129	RhV 10e3 #1	11	29	11	32	25	16	83	11	9	10	14	737
130	RhV 10e3 #2	11	29	3	27	18	10	69	11	10	4	8	835
131	RhV 10e3 #3	13	32	9	25	16	17	66	15	6	7	9	775
132	RhV 10e2 #1	9	35	12	15	12	18	56	10	6	7	9	116
133	RhV 10e2 #2	11	33	11	25	12	14	58	7	14	8	6	133
134	RhV 10e2 #3	14	23	12	16	14	23	60	11	11	9	4	104
135	RhV 10e1 #1	14	31	22	20	21	22	58	12	13	11	8	23
136	RhV 10e1 #2	5	36	17	27	22	22	69	11	5	8	9	22
137	RhV 10e1 #3	14	28	11	24	17	23	59	13	8	10	15	16
138	CVA 10e4 ctrl	16	10	6	8	12	16	15	4	12	5	11	1837
139	CVA 10e4 #1	8	22	10	17	12	10	20	10	5	5	9	1282
140	CVA 10e4 #2	9	22	6	16	7	16	27	7	10	5	10	1288
141	CVA 10e4 #3	4	20	12	30	9	12	20	5	7	7	9	966
142	CVA 10e3 #1	7	30	12	23	30	15	73	12	11	10	10	146
143	CVA 10e3 #2	1	31	9	19	25	12	61	14	0	5	6	149
144	CVA 10e3 #3	10	40	9	16	22	10	65	5	4	6	3	194
145	CVA 10e2 #1	4	16	8	14	9	16	32	14	5	3	5	18
146	CVA 10e2 #2	10	26	5	21	11	9	38	7	7	11	5	84
147	CVA 10e2 #3	14	21	6	16	17	11	35	14	2	8	11	16
148	CVA 10e1 #1	9	21	5	21	12	16	31	3	2	5	9	9
149	CVA 10e1 #2	9	33	13	15	11	18	43	6	11	10	6	9
150	CVA 10e1 #3	11	30	6	18	26	10	58	10	8	9	5	20
151	CVB 10e4 ctrl	9	15	6	10	8	13	18	11	12	10	14	2117
152	CVB 10e4 #1	8	30	4	25	26	12	41	2	2	11	12	515
153	CVB 10e4 #2	9	26	8	13	21	16	33	6	6	6	5	395
154	CVB 10e4 #3	13	30	9	12	10	13	38	8	9	5	8	144
155	CVB 10e3 #1	6	23	15	24	21	15	37	9	8	8	11	106
156	CVB 10e3 #2	10	23	6	20	12	8	23	11	2	0	6	14
157	CVB 10e3 #3	12	22	7	28	13	7	34	16	6	4	10	84
158	CVB 10e2 #1	9	23	2	16	10	6	29	8	5	6	10	20
159	CVB 10e2 #2	11	27	5	17	15	23	26	17	12	12	9	20
160	CVB 10e2 #3	4	21	13	16	9	13	28	12	12	6	12	8
161	CVB 10e1 #1	5	24	5	8	12	16	18	7	9	8	9	2
162	CVB 10e1 #2	10	16	14	23	10	17	28	10	6	4	5	7
163	CVB 10e1 #3	15	18	10	21	15	17	29	8	2	11	10	8
Mean of background		14.2	36.2	11.3	24.8	20.5	19.1	60.9	12.2	9.9	9.5	11.4	13.7
Standard deviation		7.9	12.7	4.4	7.5	9.0	6.3	23.9	4.2	4.5	4.1	4.0	6.4
Cutoff		53.5	99.5	33.6	62.2	65.7	50.9	180.2	32.9	32.4	30.0	31.5	45.7

Appendix B
Replacement Sheets 35, 36, 37, 38 and 39

Table 2- Primer sequences and detection oligonucleotide sequences used in the multiplex amplification in one embodiment of the method disclosed.

GenBank ID	Target Name	Oligonucleotides sequences	Positions	SEQ ID NO.
AY278491	SARS			
	SARS1Fo	ACCGTAGACTCATCTCTATGATG	18121-18143	SEQ ID 1
	SARS1Fi	CAGGCCACGTTTTGTCATGCGAAGCTATTCGTCACGTTCG	18201-18220	SEQ ID 2
	SARS1Ro	TTGCATTAACTCTGGTGAATTCTG	18384-18361	SEQ ID 3
	SARS1Ri	TTCTTTGCGTTATGTCTCTGCTGTAGAAAATCCTAGCTGG	18309-18290	SEQ ID 4
	SARS1De	TAGAGGGCTGTCATGCAACT	18241-18260	SEQ ID 5
	SARS2Fo	ATGCCTAACATGCTTAGGATAATG	15246-15269	SEQ ID 6
	SARS2Fi	CAGGCCACGTTTTGTCATGCTTTCTACAGGTTAGCTAACGA	15323-15343	SEQ ID 7
	SARS2Ro	TACATTGGCTGTAACAGCTTGAC	15482-15460	SEQ ID 8
	SARS2Ri	TTCTTTGCGTTATGTCTCTGAGCATAAGCAGTTGTAGCATC	15440-15420	SEQ ID 9
	SARS2De	GTGAGATGGTCATGTGTGGC	15361-15380	SEQ ID 10
	SARS3Fo	ACAATGCTGCCACCGTGCTAC	28580-28600	SEQ ID 11
	SARS3Fi	CAGGCCACGTTTTGTCATGCCCTCAAGGAACAACATTGCC	28606-28625	SEQ ID 12
	SARS3Ro	TAGCGCGAGGGCAGTTTCAC	28785-28766	SEQ ID 13
	SARS3Ri	TTCTTTGCGTTATGTCTCTGCCGCTAGCCATTCGAGCAGG	28760-28741	SEQ ID 14
	SARS3De	ATCATCACGTAGTCGCGGTAA	28681-18700	SEQ ID 15
M11486	RSVA			
	RSVAFo	ATTGGCATTAAGCCTACAAA	899-918	SEQ ID 16
	RSVAFi	CAGGCCACGTTTTGTCATGCGGGCAAATACAAAGATGGCTC	1082-1102	SEQ ID 17
	RSVARo	GACATAGCATATAACATACCTATT	1310-1287	SEQ ID 18
	RSVARi	TTCTTTGCGTTATGTCTCTGGGAGTRTCAATAYTATCTCCTGT	1202-1180	SEQ ID 19
	RSVADe	CACTCAACAAAGATCAACTT	1127-1146	SEQ ID 20
D00736	RSVB			
	RSVBFo	ATTGGCATTAAGCCTACAAA	888-907	SEQ ID 21
	RSVBFi	CAGGCCACGTTTTGTCATGCGGGCAAATACAAAGATGGCTC	1071-1091	SEQ ID 22
	RSVBRo	GACATAGCATATAACATACCTATT	1299-1276	SEQ ID 23
	RSVBRi	TTCTTTGCGTTATGTCTCTGGGAGTRTCAATAYTATCTCCTGT	1191-1169	SEQ ID 24
	RSVBDe	CATTAAATAAGGATCAGCTG	1116-1135	SEQ ID 25
AF457102	PIV-1			
	PIV1Fo	CACAATTGATATGAATTATTGG	13037-13058	SEQ ID 26
	PIV1Fi	CAGGCCACGTTTTGTCATGCTGATGAAATAATTAGAGCAACTAG	13064-13087	SEQ ID 27
	PIV1Ro	CTATTWATATCATCATCATTT	13186-13166	SEQ ID 28

	PIV1Ri	TTCTTTGCGTTATGTCTCTGTGCTATCATTTCTTTAAGATTG	13160-13139	SEQ ID 29
	PIV1De	CAGCTATGACTATTGCAGAC	13096-13115	SEQ ID 30
Z11575	PIV-3			
	PIV3Fo	CACAATTGATATGAATTATTGG	12911-12932	SEQ ID 31
	PIV3Fi	CAGGCCACGTTTTGTCATGCTACTGACATCATACATGCAATTTC	12938-12961	SEQ ID 32
	PIV3Ro	CTATTWATATCATCATCATTT	13060-13040	SEQ ID 33
	PIV3Ri	TTCTTTGCGTTATGTCTCTGTAACTATTATCTCTTTTAAATT	13035-13014	SEQ ID 34
	PIV3De	CTGCAATTACAATAGCAGAT	12970-12989	SEQ ID 35
NC_000912	M.Pneumoniae			
	MPMFo	ACCAGCATAAGAACCTCCTG	323867-323886	SEQ ID 36
	MPMFi	CAGGCCACGTTTTGTCATGCTCAAGTCACGTACTCGCCATC	323891-232911	SEQ ID 37
	MPMRo	TTAAACTGTTACTGTTGTGC	324086-324067	SEQ ID 38
	MPMRi	TTCTTTGCGTTATGTCTCTGTTTGCGAGATCTCGAGGGGTC	324039-324019	SEQ ID 39
	MPMDe	GCTGAATAAACCGGGTATTA	323961-323980	SEQ ID 40
AE001618	C.Pneumoniae			
	CPMFo	GCCTGCCCTATGAAAACGATG	6780-6800	SEQ ID 41
	CPMFi	CAGGCCACGTTTTGTCATGCCGTGATCCACACGAGTCATAC	6850-6870	SEQ ID 42
	CPMRo	TAAAGCTGCTTCGGGAACGTG	6970-6950	SEQ ID 43
	CPMRi	TTCTTTGCGTTATGTCTCTGTATCGGGGTTGTATTTCCTTC	6943-6923	SEQ ID 44
	CPMDe	ATCGGAAGTCGCTCTATCTT	6885-6904	SEQ ID 45
AY027864	Enterovirus			
	ENTVFo	CCTCCGGCCCCTGAATGCGG	1-20	SEQ ID 46
	ENTVFi	CAGGCCACGTTTTGTCATGCCCTAACTGTGGAGCACATGCC	26-46	SEQ ID 47
	ENTVRo	TGTCACCATAAGCAGCCAATG	152-132	SEQ ID 48
	ENTVRi	TTCTTTGCGTTATGTCTCTGTAGTCGGTTCCGCTGCAGAG	100-81	SEQ ID 49
	ENTVDe	CCAGAGGGTAGTGTGTCGTA	53-72	SEQ ID 50
AJ344037	InfluenzaA			
	INFAFo	TGCAATTGGGGTCCTCATCGG	528-548	SEQ ID 51
	INFAFi	CAGGCCACGTTTTGTCATGCTTGAATGGAATGATAACACAG	554-574	SEQ ID 52
	INFARo	AAACGAGAAAGTTCTTATCTC	826-806	SEQ ID 53
	INFARi	TTCTTTGCGTTATGTCTCTGGTTCTCGCCATTTTCCGTTTC	674-654	SEQ ID 54
	INFADe	TCTACAGAGATTCGCTTGG	591-609	SEQ ID 55
AF492482	InfluenzaB			
	INFBFo	TGAAGGGTTTGAGCCATACTG	291-311	SEQ ID 56
	INFBFi	CAGGCCACGTTTTGTCATGCTACAATTGGACCGATTACCCT	346-366	SEQ ID 57
	INFBRo	TGAGTGTTTACTTCCTCCTTTATC	497-474	SEQ ID 58

	INFBRi	TTCTTTGCGTTATGTCTCTGGTTGTTCATGTCCCTTAATACT	456-435	SEQ ID 59
	INFBDe	CCTTGATGACATAGAAGAAG	384-403	SEQ ID 60
AF542122	Adenovirus			
	ADVFo	AACAGACCCAAYTACATTGG	910-929	SEQ ID 61
	ADVFi	CAGGCCACGTTTTGTCATGCATGTACTACAACAGTACTGG	955-974	SEQ ID 62
	ADVRo	TATGACAGTTCWGTGTTTCTGTC	1052-1033	SEQ ID 63
	ADVRi	TTCTTTGCGTTATGTCTCTGGCAAGTCAACCACHGCATTC	1030-1011	SEQ ID 64
AY008279	ADV21De	GAGTGCTGGCAGGTCAAGCA	1034-1053	SEQ ID 65
AF542129	ADV3De	GAGTTTTGGCTGGCCAAGCA	1016-1035	SEQ ID 66 & 67
AF542122	ADV4De	GGGTACTGGCCGGTCAGGCC	983-1002	SEQ ID 68
AF515814	ADV7De	GAGTTTTGGCCGGCCAAGCA	1131-1150	SEQ ID 69
AB018425	ADV14De	GGGTGCTGGCTGGCCAAGCA	717-736	SEQ ID 70

Super primer sequences
FSP- CAGGCCACGTTTTGTCATGC SEQ ID 71
RSP- TTCTTTGCGTTATGTCTCTG SEQ ID 72

Table 3 Primer sequences and detection oligonucleotide sequences used in the multiplex amplification in one embodiment of the method disclosed.

SARS	**Sequence 5' to 3'**	**SEQ ID NO.**
SARS1Fo	ACCGTAGACTCATCTCTATGATG	SEQ ID 73
SARS1Fi	CAGGCCACGTTTTGTCATGCGAAGCTATTCGTCACGTTCG	SEQ ID 74
SARS1Ro	TTGCATTAACTCTGGTGAATTCTG	SEQ ID 75
SARS1Ri	TTCTTTGCGTTATGTCTCTGCTGTAGAAAATCCTAGCTGG	SEQ ID 76
SARS2Fo	ATGCCTAACATGCTTAGGATAATG	SEQ ID 78
SARS2Fi	CAGGCCACGTTTTGTCATGCTTTCTACAGGTTAGCTAACGA	SEQ ID 79
SARS2Ro	TACATTGGCTGTAACAGCTTGAC	SEQ ID 80
SARS2Ri	TTCTTTGCGTTATGTCTCTGAGCATAAGCAGTTGTAGCATC	SEQ ID 81
SARS4Fo	ACAATGCTGCCACCGTGCTAC	SEQ ID 83
SARS4Fi	CAGGCCACGTTTTGTCATGCCCTCAAGGAACAACATTGCC	SEQ ID 84
SARS4Ro	TAGCGCGAGGGCAGTTTCAC	SEQ ID 85
SARS4Ri	TTCTTTGCGTTATGTCTCTGCCGCTAGCCATTCGAGCAGG	SEQ ID 86
SARS1De	TAGAGGGCTGTCATGCAACT	SEQ ID 77
SARS2De	GTGAGATGGTCATGTGTGGC	SEQ ID 82
SARS4De	TCATCACGTAGTCGCGGTAA	SEQ ID 87
RSV A		
RSVAFo	AAGAATTTGATAAGTACCAC	SEQ ID 88
RSVAFi	CAGGCCACGTTTTGTCATGCACTCCCTTGGTTAGAGATGG	SEQ ID 89
RSVARi	TTCTTTGCGTTATGTCTCTGCAATGCTACTTCATCATTGTC	SEQ ID 91
RSVARo	TATGTATCACTGCCTTAGCC	SEQ ID 90
RSVADe	GCAGCAATTCATTGAGTATG	SEQ ID 92
RSV B		
RSVBFo	AATAAGAATTTGATAAGTGC	SEQ ID 93
RSVBFi	CAGGCCACGTTTTGTCATGCACCTTTTCAATCAGAAATGG	SEQ ID 94
RSVBRi	TTCTTTGCGTTATGTCTCTGCAATGCTACTTCGTCATTGTC	SEQ ID 96
RSVBRo	TGCTTTGGCTAATGCATTGG	SEQ ID 95
RSVBDe	GGTGCAATTCACTGAGCATG	SEQ ID 97
PIV1		
PIV1Fo	AGTATCACTCCTTGCAATGG	SEQ ID 98
PIV1Fi	CAGGCCACGTTTTGTCATGCATCTCACTACAAACGGTGTC	SEQ ID 99
PIV1Ri	TTCTTTGCGTTATGTCTCTGTTTGACAATGAACCCATCTG	SEQ ID 101
PIV1Ro	GTTCTTTCATACTCCATGTC	SEQ ID 100
PIV1De	GCTGATGTCAAGTATGTGAT	SEQ ID 102
PIV3		
PIV3Fo	TCAATGGCTTATGCCAATCC	SEQ ID 103
PIV3Fi	CAGGCCACGTTTTGTCATGCACAACAAATGGAAGTAATGC	SEQ ID 104
PIV3Ri	TTCTTTGCGTTATGTCTCTGCTCGTCTTAACCACAAATCC	SEQ ID 106
PIV3Ro	CAGGTCACTTCCAAATATCC	SEQ ID 105

PIV3De	CTAAAACGGCAAAAGTATGG	SEQ ID 107
InfA		
INFAFo	TGCAATTGGGGTCCTCATCGG	SEQ ID 108
INFAFi	CAGGCCACGTTTTGTCATGCTTGAATGGAATGATAACACAG	SEQ ID 109
INFARo	AAACGAGAAAGTTCTTATCTC	SEQ ID 110
INFARi	TTCTTTGCGTTATGTCTCTGGTTCTCGCCATTTTCCGTTTC	SEQ ID 111
INFADeC	TCTACAGAGATTCGCTTGG	SEQ ID 112
INFB		
INFBFo	AGTCTTATCCCAATTTGGTC	SEQ ID 113
INFBFi	CAGGCCACGTTTTGTCATGCAGAGCACCGATTATCACCAG	SEQ ID 114
INFBRi	TTCTTTGCGTTATGTCTCTGCATGTCAGCTATTATGGAGC	SEQ ID 116
INFBRo	AAGCACTGCCTGCTGTACAC	SEQ ID 115
INFBDe	TTCCACAAAACAGTAATAGC	SEQ ID 117
MPN		
MPNFo	ATCACCTTTAACCCCTTTGG	SEQ ID 118
MPNFi	CAGGCCACGTTTTGTCATGCCGGCTTTGGTTTGAGTGGG	SEQ ID 119
MPNRi	TTCTTTGCGTTATGTCTCTGCGCGGCACGAGTAAAACGGC	SEQ ID 121
MPNRo	TGCAACTGCTCATAGTACAC	SEQ ID 120
MPNDe	TGCACCCCAACAGTGAAACG	SEQ ID 122
CPN		
CPN 5 Fo	GAAATTTATAGAGCCGACTCG	SEQ ID 123
CPN 5 Fi	CAGGCCACGTTTTGTCATGCGCTGATATCATTGTACATGG	SEQ ID 124
CPN 5 Ro	GTTGACCATATAATACGTCTC	SEQ ID 125
CPN 5 Ri	TTCTTTGCGTTATGTCTCTGGCTTTCCAGGGCATTCTC	SEQ ID 126
CPN5 De	ACCGACAAAACGTAGTAACA	SEQ ID 127
ENTV		
CVA2 Fo	CAAGGTGTGAAGAGCCTATTG	SEQ ID 128
CVBEV Fo	CATGGTGCGAAGAGTCTATTG	SEQ ID 129
RhV2 Fo	GTGAAGAGCC(GC)CGTGTGCTC	SEQ ID 130
ENT3 Fi	TTCTTTGCGTTATGTCTCTGAGTCCTCCGGCCCCTGAATG	SEQ ID 131
ENT3 Ri	CAGGCCACGTTTTGTCATGCAAACACGGACACCCAAAGTAG	SEQ ID 132
CVEV Ro	ATTGTCACCATAAGCAGCC	SEQ ID 133
RhV2 Ro	TATATATTGTCACCATAAGC	SEQ ID 134
CVEV De	GTTAGGATTAGCCGCATTCA	SEQ ID 135
RhV2 De	GTTGGTCCCATCCCGCAATT	SEQ ID 136
ADV		
ADV3-3Fi	CAGGCCACGTTTTGTCATGCCCCATGGATGAGCCCACCC	SEQ ID 138
ADV3-3Ri	TTCTTTGCGTTATGTCTCTGGCTGGTGCACTCTGACCACG	SEQ ID 139
ADV4-3Ri	TTCTTTGCGTTATGTCTCTGGCTGGTGCACTCGGACGACG	SEQ ID 140

ADV14-3Ri	TTCTTTGCGTTATGTCTCTGGCTGATGCACTCTGACCACG	SEQ ID 141
ADV3-3Fo	AGCAACTTCATGTCYATGGG	SEQ ID 137
ADV3-3Ro	GTGCGCAGGTAGACGGCCTC	SEQ ID 142
ADV14-3Ro	GTACGCAGGTAGACTGTCTC	SEQ ID 143
ADV3-3De	GCTTTATCTTCTTTTCGAAG	SEQ ID 144
ADV4-3De	TCTCTATGTTGTCTTCGAAG	SEQ ID 145
ADV14-3De	GCTTTATCTTCTCTTCGAAG	SEQ ID 146
Superprimers		
FSP	CAGGCCACGTTTTGTCATGC	SEQ ID 71
RSP	TTCTTTGCGTTATGTCTCTG	SEQ ID 72